

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding a thiamin pyrophosphokinase comprising a member selected from the group consisting of:

- 5 (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8;
- (b) an isolated nucleic acid fragment that is complementary to (a).

10 2. The isolated nucleic acid fragment of Claim 1 wherein nucleic acid fragment is a functional RNA.

3. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7.

15 4. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.

5. A transformed host cell comprising the chimeric gene of Claim 4.

6. A thiamin pyrophosphokinase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8.

20 7. A method of altering the level of expression of a thiamin pyrophosphokinase in a host cell comprising:

- (a) transforming a host cell with the chimeric gene of Claim 4; and
- (b) growing the transformed host cell produced in step (a) under conditions
- 25 that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of a thiamin pyrophosphokinase in the transformed host cell.

8. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a thiamin pyrophosphokinase comprising:

- 30 (a) probing a cDNA or genomic library with the nucleic acid fragment of Claim 1;
- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of Claim 1;
- (c) isolating the DNA clone identified in step (b); and
- 35 (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding a thiamin pyrophosphokinase.

9. A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding a thiamin pyrophosphokinase comprising:

- 5 (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 5 and 7; and
(b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding a thiamin pyrophosphokinase.

10 10. The product of the method of Claim 8.

11. The product of the method of Claim 9.

12. A method for evaluating at least one compound for its ability to inhibit the activity of a thiamin pyrophosphokinase, the method comprising the steps of:

- 15 (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a thiamin pyrophosphokinase, operably linked to suitable regulatory sequences;
(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the thiamin pyrophosphokinase encoded
20 by the operably linked nucleic acid fragment in the transformed host cell;
(c) optionally purifying the thiamin pyrophosphokinase expressed by the transformed host cell;
(d) treating the thiamin pyrophosphokinase with a compound to be tested;
25 and
(e) comparing the activity of the thiamin pyrophosphokinase that has been treated with a test compound to the activity of an untreated thiamin pyrophosphokinase,

thereby selecting compounds with potential for inhibitory activity.

30
/